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TOBACCO INDUSTRY RESEARCH COMMITTEE

150 East Forty Second Street

New York 17, N.Y.

Application for Research Grant

Date: January 31, 1962

1. Name of Investigator: E. T. ANGELAKOS, M. D., PH.D.
2. Title: Associate Professor of Physiology
3. Institution & Address: Boston University School of Medicine
Department of Physiology
80 East Concord Street
Boston, Massachusetts
4. Project or Subject: "RELEASE OF CATECHOL AMINES FROM THE ISOLATED HEART"
5. Detailed Plan of Procedure:

There is now ample evidence that within the connective tissue of the heart there is a significant number of chromaffin cells which in all probability contain appreciable quantities of catechol amines especially epinephrine (1, 3, 4, 6). The role of the possible liberation of catechol amines in cardiac physiology is at present obscure. However, there is evidence that catechol amines are liberated following an adequate stimulus. Notably larger amounts of acetylcholine (ACh) have been shown to produce catechol amine liberation in the heart (1,2).

The purpose of the proposed investigation is to study the ability of certain compounds to release catechol amines from the isolated heart in vitro. It is anticipated that such a study will provide some insight and in particular it will serve to elucidate the pharmacological characteristics of catechol amine liberation. Information about catechol liberators will be of particular value since such substances may serve as "pharmacological tools" in future studies.

There is some suggestive evidence that the cardiac chromaffin tissue may be physiologically and pharmacologically similar to the adrenal medulla and hence to be akin to autonomic ganglia. In this connection there is a direct relation to nicotine which exhibits both ganglionic stimulant and depressant properties and will be used in this study for this purpose. In fact the pharmacological characteristics of catechol amine liberation by ACh are such, that this action of ACh has been classified as "nicotinic" (1).

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The methods to be used are relatively simple and some of them have been in constant use in this laboratory for some time. The isolated preparations will include rabbit, guinea pig and cat atria and rabbit and rat hearts perfused with ionically balanced buffered solutions. Determination of catechol amines will be made in the effluent. Catechol amine isolation, determination and separation will be made using the recently developed techniques. This will include adsorption on alumina, elution, oxidation and determination of the fluorescent product with an ultraviolet fluorometer (5,6). Paper chromatograms will also be studied.

Mechanical and electrical responses of all the preparations will be continuously recorded through special amplifiers, transducers and recorders now available and in operation in this laboratory. Thus it will be possible to determine in addition the immediate mechanical and electrical manifestations of catechol amine liberation.

In controlled experiments reserpine and similar compounds will be used to deplete the catechol amine stores and comparison will be made between normal and depleted hearts. Furthermore, inhibitors of the known catechol metabolizing enzymes (O-methyl-transferase and mono-amine oxydase) will be used in vivo and in vitro to increase the catechol amine content and recovery of released amines (7). A perfusate rich in ascorbic acid will be used in all cases to reduce the rate of autoxidation of released catechols.

Preliminary plans have been made so that the early part of this study may be conducted in Dr. von Euler's laboratory (Karolinska Institute, Sweden) where the principal investigator plans to spend a period of time in order to become more familiar with current techniques of catechol amine identification and measurement, and to study various other aspects of catechol amine release. Dr. von Euler's group is now actively engaged in the problem of catechol amine liberation and a collaboration with that group during the early phases of the proposed project would be most fruitful. Part of the funds to cover the principal investigators trip to Sweden will be provided for by a Career Development Award Grant from the U.S. Public Health Service to the principal investigator.

R E F E R E N C E S

1. Hoffman F., Hoffman, E. J., Middleton, S. and Talesnik, J., The Stimulating Effect of Acetylcholine on the Mammalian Heart and the Liberation of an Epinephrine Like Substance by the Isolated Heart, *Am. J. Physiol.*, 144:189, 1945.
2. Ginzell and Kottegoda, S.R., Nicotine-like Action in Auricles and Blood Vessels after Denervation, *Brit. J. Pharmacol.*, 8:348, 1953.
3. Raab, W. and Gigg, W., Norepinephrine and Epinephrine Content of Normal and Diseased Human Hearts, *Circulation*, 11:593, 1955.

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4. Day, M. The Release of Substances Like Acetylcholine and Adrenaline by the Isolated Rabbit Heart, J. Physiol., 134:558, 1956.
5. Underfriend S., Survey of Chemical and Physical Methods for Measuring Physiological Amounts of Catechol Amines in Symposium on Catechol amines, Pharm. & Exp. Therap. Study Section NIH, 1958.
6. von Euler, U.S. Autonomic Neuroeffector Transmission in Handbook of Physiology, I:215, 1959.
7. Crout, J. R., Creveling, C. R. and Underfriend S., Norepinephrine Metabolism in the Rat Brain and Heart, J. Pharm. Exp. Therap. 132:269, 1961.

ITEMIZED BUDGET

	<u>1962-63</u>	<u>1963-64</u>
<u>Salaries</u>		
Technical Assistance (1)	\$4,500.00	\$4,500.00
Fringe Benefits (7.5%) (2)	238.00	238.00
<u>Permanent Equipment</u>		
Turner Fluorometer (3) (with accessories)	2,000.00	
<u>Supplies</u>		
Chemical & Laboratory Suppl. (1)	800.00	800.00
Animals	300.00	300.00
<u>Travel</u> (4)	600.00	200.00
<u>Reprints & Publ. Costs</u>	250.00	250.00
	<u>\$8,688.00</u>	<u>\$6,288.00</u>

TOTAL FOR TWO YEARS: \$14,976

(1) Authorization is requested that part of the above funds for technical assistance and/or laboratory supplies may be used at the Karolinska Institute in Sweden (Dr. von Euler's Laboratory). Boston University would be authorized to make advances and/or reimbursements for such expenditures.

(2) According to general Boston University Policy.

(3) This instrument is essential for the fluorimetric determinations of catechol amines.

(4) It is also requested that the budgeted travel funds may be used to defray travel expenses connected with the visit and stay in Sweden of the principal

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investigator. However, part of this cost will be provided by another source. (Career Development Fellowship Grant by USPHS).

It is understood that all funds expended in USA or Sweden will be in connection with the above outlined project and specific accounts of all expenditures will be given.

(Based on a two year program)

6. <u>Budget Plan:</u>	a. Salaries	\$9,476.00
	b. Expendable Supplies	2,200.00
	c. Permanent Equipment	2,000.00
	d. Overhead (15% of a,b,e)	1,946.00
	e. Other	1,300.00
	<u>TOTAL</u>	<u>\$16,922.00</u>

7. Anticipated Duration of Work: June 1, 1962 to May 31, 1964

8. Facilities and Staff Available: All the laboratory facilities necessary for the performance of the proposed studies are available. This includes laboratory space, animal quarters and a large number of electronic equipment necessary for measuring electrophysiological and contractile responses in isolated hearts.

9. Additional Requirements: Funds are requested for full time technical assistance to be used in connection with this project and for supplies. The only major item needed is a Turner Fluorometer for the determination of catechol amines.

10. Additional Information (including relation of work to other projects and other sources of supply)

This project is an outgrowth of studies now in progress on the excitatory effects of acetylcholine in the heart (Supported by the American Heart Association). No source of support is available at present for the study of the specific problem of catechol amines release. The results of this project will be correlated with other studies on the electrophysiology of the heart performed in this laboratory under the direction of the principal investigator.

Signature E. T. ANGELAKOS, M. D. PH.D.
Director of Project

Kurt M. Hertzfeld
Business Officer of the Institution

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